



Original research article

Microgreens of Brassicaceae: Mineral composition and content of 30 varieties

Zhenlei Xiao^{a,b}, Eton E. Codling^c, Yaguang Luo^{a,*}, Xiangwu Nou^d, Gene E. Lester^a, Qin Wang^b^a Food Quality Laboratory, USDA Agricultural Research Service, Beltsville, MD 20705, United States^b Department of Nutrition and Food Science, University of Maryland, College Park, MD 20740, United States^c Crop Systems and Global Change Laboratory, USDA Agricultural Research Service, Beltsville, MD 20705, United States^d Environmental Microbial and Food Safety Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705, United States

ARTICLE INFO

Article history:

Received 25 December 2015

Received in revised form 6 April 2016

Accepted 19 April 2016

Available online 22 April 2016

Keywords:

Microgreens

Brassicaceae

Food composition

Macroelements

Microelements

ICP OES

Hierarchical cluster analysis

ABSTRACT

The mineral element composition was analyzed for 30 varieties of microgreens, representing 10 species within 6 genera of the Brassicaceae family. Brassicaceae microgreens were assayed for concentrations of macroelements, including calcium (Ca), magnesium (Mg), phosphorous (P), sodium (Na), potassium (K), and of microelements, including copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Determinations of mineral elements in microgreen samples were performed using an inductively coupled plasma optical emission spectrophotometer (ICP OES). Potassium was the most abundant macroelement ranging from 176 to 387 mg/100 g fresh weight (FW), followed by P (52–86 mg/100 g FW), Ca (28–66 mg/100 g FW), Mg (28–66 mg/100 g FW), and Na (19–68 mg/100 g FW). Among the microelements, Fe tended to be most abundant (0.47–0.84 mg/100 g FW), followed by Zn (0.22–0.51 mg/100 g FW), Mn (0.17–0.48 mg/100 g FW), and Cu (0.041–0.13 mg/100 g FW). Based upon the analysis of 30 varieties, the results demonstrate that microgreens are good sources of both macroelements (K and Ca) and microelements (Fe and Zn). Consumption of microgreens could be a health-promoting strategy to meet dietary reference intake requirements for essential elements beneficial to human health.

Published by Elsevier Inc.

1. Introduction

Essential mineral elements are a class of nutritionally important nutrients for human health, which are best obtained from dietary sources (Martinez-Ballesta et al., 2010). They are divided into two groups: the macroelements (such as Ca, Mg, P, K, and Na) and microelements (also known as trace elements, such as Fe, Zn, Cu, and Mn), both of which play crucial roles in various biological processes for both plants (Maathuis, 2009) and the human beings (Scherz and Kirchoff, 2006). For humans, deficiencies in these elements can cause metabolic disorders and organ damage, leading to acute and chronic diseases and even death (dos Santos et al., 2013). For example, the importance of calcium and vitamin D on bone mass and bone metabolism is well known and studies have shown that adequate intake of magnesium and potassium from a healthy diet could contribute to optimize bone health, while deficiencies in these elements can cause dwarfism in children and

osteoporosis in the elderly (Macdonald et al., 2005; Nieves, 2005). Similarly, iron is vital to biological functions, including respiration, energy production, DNA synthesis, and cell proliferation (Hentze et al., 2010). Inadequate intake of iron is the leading cause of anemia, which remains a global health issue, affecting people of all ages (Camaschella, 2015). Adequate dietary intake of mineral elements is necessary for human health and wellness. Unfortunately, mineral malnutrition is still a common problem worldwide and considered one of the most important global challenges for human nutrition (Pinto et al., 2015).

Fruits and vegetables are rich sources of vitamins, minerals, dietary fibers and other phytochemicals, which play important roles in human nutrition. Epidemiological and clinical studies have indicated an inverse association between increased consumption of fruits and vegetable and the occurrence of certain chronic diseases, such as cardiovascular diseases, cancers and some degenerative diseases, such as osteoporosis and cataracts (Boeing et al., 2012; Hussain et al., 2014).

Microgreens are an emerging class of specialty fresh produce, which have gained increasing popularity with chefs and consumers in recent years. They are young seedlings of vegetables and

* Corresponding author.

E-mail address: yaguang.luo@ars.usda.gov (Y. Luo).

herbs, harvested when cotyledons are fully developed and the first pair of true leaves are emerging or partially expanded. Previous studies have shown that microgreens are good sources of vitamins and other phytonutrients, such as carotenoids and polyphenols (Sun et al., 2013; Xiao et al., 2012) Owing to their nutrient-dense properties, microgreens have recently attracted considerable attention from researchers of human nutrition, public health professionals and educators, and health-conscious consumers. The mineral composition of microgreens has rarely been reported. Kopsell and Sams (2013) investigated the changes in shoot tissue pigments, glucosinolates and mineral elements of broccoli microgreens exposed to short-duration blue light from light-emitting diodes. Kou et al. (2014) found that pre-harvest application of calcium improved post-harvest quality and shelflife of broccoli microgreens, and compared calcium contents in treated and untreated samples. Pinto et al. (2015) and colleagues compared the mineral profiles and nitrate contents of lettuce microgreens and mature lettuces. However, all of these studies focused on an individual microgreen variety and there has not been a comprehensive study on the mineral profiles of multiple varieties of microgreens.

Brassica vegetables (such as broccoli, cabbage, and radish) are well recognized and valued for containing significant amounts of cancer-fighting glucosinolates as well as carotenoid phytochemicals, vitamins and mineral elements (Kopsell and Sams, 2013). The presence and abundance of essential mineral elements is an important criterion for the assessment of nutritional quality and influence on human health and have value for both consumers and public health professionals.

This study reports mineral composition data including calcium (Ca), copper (Cu), iron (Fe), potassium (K), sodium (Na), magnesium (Mg), manganese (Mn), phosphorous (P), and zinc (Zn), as well as heavy metal toxins cadmium (Cd) and lead (Pb) for 30 of the most common commercial varieties of Brassica microgreens using inductively coupled plasma optical emission spectrometry (ICP

OES). The data were evaluated using the multivariate analysis technique, as well as by hierarchical cluster analysis (HCA).

2. Materials and methods

2.1. Sample collection and preparation

In total, 30 varieties of microgreens representing 6 Brassicaceae genera (Table 1) were obtained from Fresh Origins Farm (San Marcos, CA, USA), between March to May 2014. All the microgreens were grown in peat moss substrata in unheated greenhouses, under ambient light and temperature. Microgreen samples were harvested without seed coats or roots. Samples were immediately packed in clamshell containers and shipped overnight in a cardboard box filled with insulating foam and ice packs to maintain a cold temperature. Upon receiving product at the USDA-ARS Beltsville Agricultural Research Center (Beltsville, MD, USA), microgreen samples were inspected and any individual microgreen with visible defect was discarded. Fifty grams of each sample was collected and rinsed three times under tap water followed by a single deionized water rinse to remove any surface residue. After washing, samples were flash frozen in liquid nitrogen and lyophilized for 48–72 h. The commercial names, scientific names and growth period (days) of the 30 varieties of Brassicaceae microgreens are listed in Table 1.

2.2. Reagents and standards

All chemical reagents used in this study were of analytical grade and obtained from Thermo Fisher Scientific Inc. (Frederick, MD, USA). Deionized water was used to prepare all solutions. Standard stock solutions were prepared in 2% (v/v) nitric acid (HNO₃) with the concentration of 10,000 mg/L for elements Ca and K, and 1000 mg/L for elements Mg, P, Na, Fe, Zn, Cu, and Mn, respectively. SPEX CertiPrep (Metuchen, NJ, USA) certified reference materials

Table 1
Product information of 30 commercially grown microgreens in Brassicaceae family assayed in this study.

Commercial name	Scientific name (genus and species)	Growth length (day)	Dry weight percentage (%)
Arugula	<i>Eruca sativa</i> Mill.	7	6.4 ± 0.1
Broccoli	<i>Brassica oleracea</i> L. var. <i>italica</i>	9	7.9 ± 0.1
Brussel sprouts	<i>Brassica oleracea</i> L. var. <i>Gemmifera</i>	9	6.5 ± 0.1
Cabbage Chinese	<i>Brassica rapa</i> L. var. <i>pekinensis</i>	6	5.2 ± 0.0
Cabbage green	<i>Brassica oleracea</i> L. var. <i>capitata</i> f. <i>alba</i>	9	6.1 ± 0.0
Cabbage red	<i>Brassica oleracea</i> L. var. <i>capitata</i> f. <i>rubra</i>	7	5.9 ± 0.0
Cabbage savoy	<i>Brassica oleracea</i> L. var. <i>capitata</i> f. <i>sabauda</i>	9	7.0 ± 0.0
Cauliflower	<i>Brassica oleracea</i> L. var. <i>botrytis</i>	9	6.8 ± 0.0
Collard	<i>Brassica oleracea</i> L. var. <i>viridis</i>	10	7.0 ± 0.2
Kale Chinese	<i>Brassica oleracea</i> L. var. <i>alboglabra</i>	8	7.5 ± 0.0
Kale red	<i>Brassica oleracea</i> L. var. <i>acephala</i>	9	5.6 ± 0.1
Kale Tuksan	<i>Brassica oleracea</i> L. var. <i>acephala</i>	9	7.5 ± 0.1
Kohlrabi purple	<i>Brassica oleracea</i> L. var. <i>gongylodes</i>	8	8.0 ± 0.4
Komatsuna red	<i>Brassica rapa</i> L. var. <i>perviridis</i>	8	6.7 ± 0.2
Mizuna	<i>Brassica rapa</i> L. var. <i>nipposinica</i>	8	5.5 ± 0.0
Mustard Dijon	<i>Brassica juncea</i> (L.) Czern.	9	6.1 ± 0.1
Mustard red	<i>Brassica juncea</i> (L.) Czern.	9	4.9 ± 0.1
Pak choy	<i>Brassica rapa</i> L. var. <i>chinensis</i>	8	5.0 ± 0.0
Peppercress	<i>Lepidium bonariense</i> L.	6	5.7 ± 0.4
Radish China rose	<i>Raphanus sativus</i> L.	9	6.8 ± 0.1
Radish daikon	<i>Raphanus sativus</i> L. var. <i>longipinnatus</i>	8	6.7 ± 0.4
Radish red	<i>Raphanus sativus</i> L.	7	6.9 ± 0.1
Radish ruby	<i>Raphanus sativus</i> L.	5	10.3 ± 0.1
Rapini	<i>Brassica rapa</i> L. var. <i>ruvo</i>	8	8.4 ± 0.3
Rutabaga	<i>Brassica napus</i> L. var. <i>napobrassica</i>	9	5.9 ± 0.1
Tatsoi	<i>Brassica narinosa</i> L. var. <i>rosularis</i>	7	5.8 ± 0.0
Turnip	<i>Brassica rapa</i> L. var. <i>rapa</i>	9	5.3 ± 0.1
Upland cress	<i>Barbarea verna</i> (P. Mill.) Aschers	20	7.2 ± 0.2
Wasabi	<i>Wasabia japonica</i> Matsum.	7	6.8 ± 0.1
Watercress	<i>Nasturtium officinale</i> L.	17	6.2 ± 0.2

were used to prepare working standard solutions by diluting with 1 mol/L nitric acid. All laboratory glassware used were decontaminated by submerging them in a sulfuric acid solution (10%, v/v) for 24 h, and then rinsed with deionized water three times before use.

2.3. Sample digestion

The lyophilized microgreen tissue (1.5–2.0 g) was ashed in a muffle furnace at 480 °C for 16 h. The ash was further digested using 2 mL concentrated nitric acid (HNO₃, 65%, v/v) on a hot plate. After drying, 10 mL of 3 mol/L hydrochloric acid (HCl) was added to each sample and allowed to flux for 2 h. Subsequently, the digest was filtered using Whatman #40 filter paper and the filtrate was diluted to a volume of 25 mL with 0.1 mol/L HCl (AOAC, 1984).

2.4. Sample analysis

Concentrations of Ca, Mg K, P, Na Cu, Fe Mn, and Zn in the solutions of the digested samples were determined using a Perkin-Elmer DV 4300 inductively coupled plasma optical emission spectrophotometer (ICP OES, Norwalk, CT, USA) with axial viewing and a segmented-array detector (SCD). A Cyclone spray chamber and a Mira-mist nebulizer were also utilized. Scandium (15 µg/L) was used as internal standard for all determinations. The instrumental parameters are shown in Table 2.

The accuracy of the method was evaluated using a certified reference material: spinach leaves (NIST 1570a), (National Institute of Standards and Technology, Gaithersburg, MD, USA). The certified material was digested using the same procedure employed for the microgreen samples. For quality assurance, one blank and one spinach leaf standard were included for every 20 samples analyzed. The analytical results obtained in this study were in agreement with the certified values (expressed as dry weight). The limit of detection (LOD) and limit of quantification (LOQ) were both calculated by a background equivalent concentration (BEC) test using the following formulas: $LOD = 3 * BEC * \text{relative standard deviation of 10 blanks (RSD}_0)$; $LOQ = 10 * BEC * \text{relative standard deviation of 10 blanks (RSD}_0)$.

2.5. Statistical analysis

In this study, mineral composition analyses were performed on three replicate samples, which were harvested from three different locations in the same greenhouse on the same day. Mineral element concentrations were calculated on a fresh weight basis and data were expressed as mean ± standard error. The differences

Table 2

Instrumental parameters used in the inductively coupled plasma optical emission spectrometry (ICP OES) in this study.

Parameters	Conditions	
Radio frequency generator (MHz)	40	
Radio frequency power (kW)	1.5	
Plasma gas rate (L min ⁻¹)	15.0	
Auxiliary gas rate (L min ⁻¹)	0.2	
Nebulizer gas rate (L min ⁻¹)	0.6	
Mist chamber	Cyclone	
Nebulizer	Mira-mist	
Injector tube diameter (mm)	2.0	
Spray chamber	Cyclonic	
Nebulizer	Gem Cone	
Emission lines (nm)	Ca(II) 315.887	Fe(II) 238.204
	Mg(II) 279.077	Zn(II) 206.200
	P(I) 214.914	Cu(I) 324.752
	K(II) 404.721	Mn(II) 257.610
	Na(I) 589.592	

Table 3A

Results observed for the certified reference materials spinach leaves NIST 1570a.

Macro-Element	Certified value (%)	Measured value
Ca	1.527 ± 0.041	1.310 ± 0.100
K	2.903 ± 0.052	2.896 ± 0.320
Mg	0.890 NC ^a	0.746 ± 0.050
P	0.518 ± 0.011	0.509 ± 0.050
Na	1.818 ± 0.430	1.685 ± 0.230
Micro-Element	(mg/kg DW)	
Fe	NV ^b	218.2 ± 15.0
Cu	12.2 ± 0.6	11.3 ± 1.1
Mn	75.9 ± 1.9	58.5 ± 3.6
Zn	82.0 ± 3.0	66.0 ± 5.0

^a NV = No values.

^b NC = Not certified.

among mineral contents of the samples were tested by one-way analysis of variance (ANOVA) using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Mean comparisons were evaluated using Tukey's honestly significant difference (HSD) test with P value of 0.05 (n = 3).

In order to extrapolate the similarities and the dissimilarities among the 10 microgreen species in mineral composition, hierarchical cluster analysis was performed on the normalized data sets (the total amounts of 9 mineral components in respective varieties of microgreens) using PROC CLUSTER (SAS version 9.3, SAS Institute Inc, Cary, NC, USA). Two clustering methods were used, namely, average-linkage method and Ward's minimum variance method. Dendrograms were produced based on the HCA results using PROC TREE (SAS version 9.3).

3. Results and discussion

3.1. Determination of mineral element composition of microgreens

The accuracy of the method was evaluated using spinach leaves NIST 1570a (Table 3A). Instrumental LOD (−0.004–20.039 mg/kg dry weight (DW)) and LOQ (−0.013–66.797 mg/kg (DW)) for each element analyses were shown in Table 3B.

Mineral macro- and microelements, including calcium (Ca), copper (Cu), iron (Fe), potassium (K), sodium (Na), magnesium (Mg), manganese (Mn), phosphorous (P), and zinc (Zn) were determined in 30 varieties of Brassicaceae microgreen samples (Tables 4 and 5). The concentrations of macroelements in

Table 3B

Limit of detection (LOD) and limit of quantification (LOQ) based on background equivalent concentration (BEC).

Macro-element	LOD (mg/kg DW)	LOQ (mg/kg DW)
Ca	0.009	0.029
K	20.039	66.797
Mg	0.002	0.005
P	−0.004	−0.013
Na	0.043	0.142
Micro-element	(mg/kg DW)	(mg/kg DW)
Fe	−0.002	−0.006
Cu	0.002	0.005
Mn	0.000	0.000
Zn	0.001	0.003
Cd	0.000	0.000
Pb	0.001	0.005

Table 4
Mean macroelement concentrations of Ca, Mg, P, K and Na in 30 commercially grown microgreens in Brassicaceae family.

Commercial name	(mg/100 g FW)				
	Ca	Mg	P	K	Na
Arugula	67 ± 2 ^a	41 ± 1	63 ± 1	343 ± 13	35 ± 1
Broccoli	88 ± 2	51 ± 1	69 ± 0	326 ± 9	52 ± 2
Brussel sprouts	81 ± 4	49 ± 1	57 ± 1	293 ± 5	54 ± 3
Cabbage Chinese	68 ± 1	31 ± 0	69 ± 1	240 ± 35	25 ± 1
Cabbage green	92 ± 1	55 ± 1	57 ± 1	192 ± 2	57 ± 1
Cabbage red	75 ± 1	39 ± 0	65 ± 0	240 ± 2	32 ± 1
Cabbage savoy	98 ± 3	62 ± 2	59 ± 2	238 ± 9	65 ± 3
Cauliflower	94 ± 2	66 ± 1	62 ± 0	224 ± 13	61 ± 1
Collard	71 ± 3	53 ± 1	75 ± 2	266 ± 5	44 ± 1
Kale Chinese	66 ± 1	45 ± 1	68 ± 0	246 ± 23	38 ± 3
Kale red	59 ± 3	36 ± 1	60 ± 1	332 ± 18	43 ± 3
Kale Tucsan	80 ± 1	52 ± 2	69 ± 2	283 ± 8	51 ± 2
Kohlrabi purple	92 ± 5	55 ± 2	77 ± 3	342 ± 7	50 ± 3
Komatsuna red	55 ± 1	40 ± 2	70 ± 4	357 ± 3	32 ± 2
Mizuna	48 ± 5	29 ± 0	57 ± 1	354 ± 7	35 ± 4
Mustard Dijon	51 ± 4	35 ± 2	62 ± 2	365 ± 32	35 ± 1
Mustard red	47 ± 2	28 ± 1	52 ± 2	289 ± 5	27 ± 1
Pak choy	58 ± 1	31 ± 2	59 ± 4	284 ± 12	42 ± 5
Peppercress	39 ± 1	33 ± 2	58 ± 4	320 ± 26	29 ± 1
Radish China rose	54 ± 2	48 ± 2	71 ± 3	270 ± 7	38 ± 2
Radish daikon	66 ± 2	60 ± 3	86 ± 4	176 ± 10	57 ± 4
Radish red	56 ± 3	49 ± 2	81 ± 2	283 ± 10	42 ± 1
Radish ruby	41 ± 3	39 ± 2	82 ± 3	215 ± 6	19 ± 0
Rapini	92 ± 2	55 ± 1	85 ± 2	359 ± 1	56 ± 2
Rutabaga	59 ± 2	44 ± 1	64 ± 1	270 ± 14	39 ± 4
Tatsoi	62 ± 2	33 ± 1	66 ± 1	329 ± 13	36 ± 1
Turnip	57 ± 3	32 ± 1	61 ± 1	341 ± 12	26 ± 1
Upland cress	79 ± 5	47 ± 2	56 ± 0	376 ± 9	35 ± 1
Wasabi	56 ± 3	41 ± 1	69 ± 3	387 ± 9	33 ± 5
Watercress	51 ± 2	32 ± 2	62 ± 2	360 ± 3	68 ± 4

^a Values are expressed as mean ± standard error (n = 3).

microgreens samples varied with species and varieties (Table 4). The most abundant macroelement in all the microgreen samples was K, followed by P, Ca, Mg and Na. Potassium was found in highest concentration in wasabi microgreens (387 mg/100 g FW), while the lowest values of K was in daikon radish microgreens (176 mg/100 g FW). The P concentration of samples analyzed in this study ranged from 52 mg/100 g FW (red mustard microgreens) to 86 mg/100 g FW (daikon radish microgreens). Ca concentration in the 30 microgreen samples varied, with the highest value in samples of savoy cabbage microgreens (98 mg/100 g FW) and the lowest in peppercress microgreens (39 mg/100 g FW). The concentration of Mg ranged from 28 to 66 mg/100 g FW with the highest value in samples of cauliflower microgreens (66 mg/100 g FW) and the lowest in red mustard microgreens (28 mg/100 g FW). Sodium concentration was highest in watercress microgreens (68 mg/100 g FW) and lowest in ruby radish microgreens (19 mg/100 g FW). Overall, the macroelements (Ca, Mg, P, K, and Na) represented 99.7 ± 0.0% of the mineral element content determined and potassium was the principal element found in all samples (57.1 ± 1.4%). Phosphorus was the second most abundant element in approximately 66% of the samples. Ca concentration exceeded that of P in the remaining 33% of samples.

Microelements in the 30 microgreen varieties also showed significant variation among different varieties (Table 5) and the microelement concentrations of Fe, Zn, Cu and Mn ranged from 0.47–0.84 mg/100 g FW, 0.22–0.51 mg/100 g FW, 0.04–0.13 mg/100 g FW, and 0.17–0.48 mg/100 g FW, respectively. In most cases, Fe content was approximately 50% of the total microelement content determined (45.1 ± 0.7%) and was highest in rapini microgreens, followed by purple kohlrabi and red komatsuna microgreens. The highest concentrations of Zn and Cu were also

Table 5
Mean concentrations of microelementary minerals in 30 commercially grown microgreens in Brassicaceae family.

Commercial name	(mg/100 g FW)					
	Fe	Zn	Cu	Mn	Cd	Pb
Arugula	0.71 ± 0.01 ^a	0.35 ± 0.01	0.07 ± 0.00	0.29 ± 0.01	ND ^b	ND
Broccoli	0.67 ± 0.01	0.37 ± 0.00	0.09 ± 0.00	0.37 ± 0.00	ND	ND
Brussel sprouts	0.57 ± 0.01	0.29 ± 0.01	0.08 ± 0.00	0.37 ± 0.01	ND	ND
Cabbage Chinese	0.66 ± 0.01	0.36 ± 0.01	0.04 ± 0.00	0.30 ± 0.01	ND	ND
Cabbage green	0.59 ± 0.01	0.29 ± 0.01	0.05 ± 0.00	0.33 ± 0.00	ND	ND
Cabbage red	0.62 ± 0.01	0.36 ± 0.01	0.08 ± 0.00	0.31 ± 0.00	ND	ND
Cabbage savoy	0.57 ± 0.02	0.27 ± 0.01	0.05 ± 0.00	0.38 ± 0.01	ND	ND
Cauliflower	0.62 ± 0.01	0.29 ± 0.01	0.06 ± 0.00	0.31 ± 0.00	ND	ND
Collard	0.65 ± 0.01	0.40 ± 0.01	0.09 ± 0.00	0.36 ± 0.01	ND	ND
Kale Chinese	0.64 ± 0.04	0.37 ± 0.01	0.06 ± 0.00	0.28 ± 0.01	ND	ND
Kale red	0.47 ± 0.01	0.29 ± 0.01	0.06 ± 0.00	0.32 ± 0.01	ND	ND
Kale Tucsan	0.72 ± 0.02	0.37 ± 0.00	0.09 ± 0.00	0.44 ± 0.01	ND	ND
Kohlrabi purple	0.75 ± 0.03	0.43 ± 0.02	0.11 ± 0.01	0.39 ± 0.01	ND	ND
Komatsuna red	0.74 ± 0.05	0.38 ± 0.02	0.08 ± 0.01	0.34 ± 0.01	ND	ND
Mizuna	0.57 ± 0.02	0.28 ± 0.01	0.06 ± 0.00	0.36 ± 0.01	ND	ND
Mustard Dijon	0.56 ± 0.01	0.29 ± 0.01	0.06 ± 0.00	0.39 ± 0.01	ND	ND
Mustard red	0.62 ± 0.07	0.22 ± 0.01	0.06 ± 0.00	0.24 ± 0.00	ND	ND
Pak choy	0.49 ± 0.02	0.30 ± 0.01	0.05 ± 0.01	0.29 ± 0.02	ND	ND
Peppercress	0.48 ± 0.03	0.41 ± 0.03	0.06 ± 0.01	0.24 ± 0.02	ND	ND
Radish China rose	0.62 ± 0.03	0.35 ± 0.01	0.08 ± 0.00	0.27 ± 0.01	ND	ND
Radish daikon	0.57 ± 0.02	0.28 ± 0.01	0.05 ± 0.00	0.19 ± 0.00	ND	ND
Radish red	0.67 ± 0.02	0.43 ± 0.00	0.11 ± 0.01	0.30 ± 0.00	ND	ND
Radish ruby	0.55 ± 0.01	0.40 ± 0.01	0.06 ± 0.00	0.17 ± 0.00	ND	ND
Rapini	0.84 ± 0.05	0.51 ± 0.02	0.13 ± 0.01	0.40 ± 0.00	ND	ND
Rutabaga	0.52 ± 0.02	0.26 ± 0.01	0.07 ± 0.00	0.39 ± 0.00	ND	ND
Tatsoi	0.57 ± 0.01	0.35 ± 0.01	0.05 ± 0.00	0.29 ± 0.01	ND	ND
Turnip	0.58 ± 0.01	0.34 ± 0.01	0.07 ± 0.00	0.41 ± 0.00	ND	ND
Upland cress	0.63 ± 0.01	0.41 ± 0.01	0.12 ± 0.00	0.48 ± 0.01	ND	ND
Wasabi	0.65 ± 0.05	0.42 ± 0.01	0.08 ± 0.00	0.26 ± 0.01	ND	ND
Watercress	0.52 ± 0.01	0.41 ± 0.02	0.09 ± 0.01	0.39 ± 0.01	ND	ND

^a Values are expressed as mean ± standard error (n = 3).

^b ND means the heavy metals (Cd and Pb) were not detected (namely, under the limit of detection, which were 0.000 and 0.001 mg/kg dry weight for Cd and Pb, respectively) in this assay.

found in rapini microgreens. Additionally, upland cress microgreens exhibited the highest concentration of Mn. At the other extreme, red kale, red mustard, Chinese cabbage and ruby radish microgreens showed the lowest values of Fe, Zn, Cu and Mn,

respectively. In addition, we assayed the microgreen samples for the toxic heavy metals (Cd and Pb). Neither Cd nor Pb were detected, (the limits of detection were 0.010 and 0.015 mg/kg, respectively), indicating the insignificance of concerns over heavy

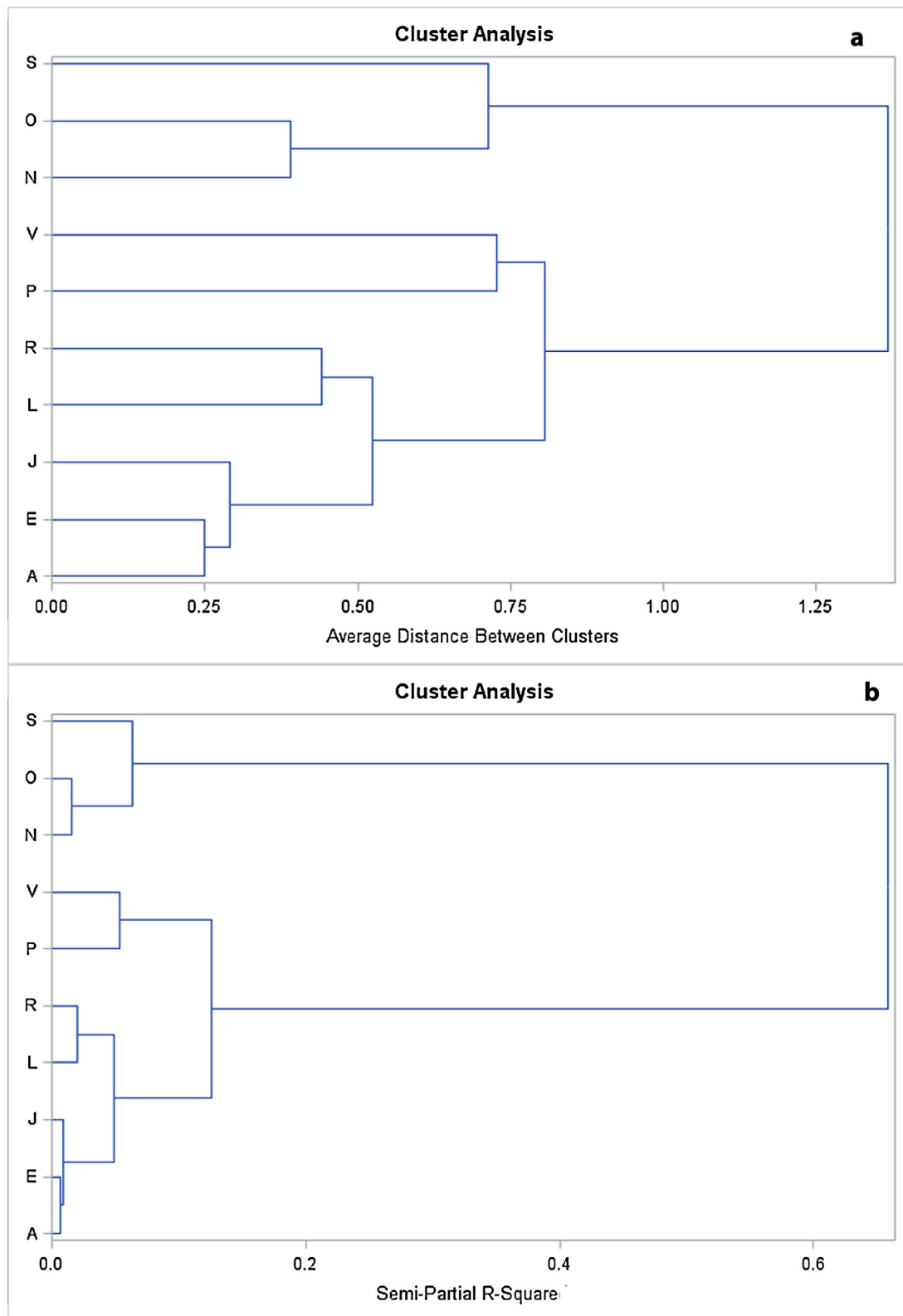


Fig. 1. The average-linkage on the normalized data sets of mineral components by means of the Hierarchical method (a) and Ward's minimum variance using Euclidean distance as measure of similarity among species (b). A = *Brassica narinosa*; E = *Eruca sativa*; J = *Brassica juncea*; L = *Lepidium bonariense*; N = *Brassica napus*; O = *Brassica oleracea*; P = *Nasturtium officinale*; R = *Brassica rapa*; S = *Raphanus sativus*; V = *Barbarea verna*.

metals in microgreens grown and harvested under typical commercial production conditions.

It is worth noting that rapini microgreens contained the highest amounts of Fe, Zn and Cu, as well as very high concentrations of Ca, Mg, P, K, Na, and Mn. In addition, the samples of purple kohlrabi microgreens also showed very high values of all microelements and macroelements. In contrast, the red mustard microgreen samples were observed to have the lowest contents of Mg, P and Zn, as well as relatively low amounts of Ca, K, Fe and Cu. Ruby radish microgreens contained considerable amounts of P and Zn, but low amounts of Na, Mn, Ca, K, Fe and Cu. Among the 30 varieties of microgreen samples, rapini and ruby radish microgreens represented the varieties with the highest and lowest overall mineral element contents (649 ± 6 mg/100 g FW and 398 ± 13 mg/100 g FW), respectively.

Little published data is available on mineral composition of microgreens, thus limiting data comparison. In general, the mineral element contents of the tested microgreen samples showed very similar profiles described for more mature plants in most of the Brassicaceae family, including broccoli, cauliflower, watercress, kale, cabbage and radish assayed in previous studies (Kawashima and Valente Soares, 2003; Sanchez-Castillo et al., 1998; Santos et al., 2014; Souza and El-Aal, 2007). Santos et al. (2014) quantified nine elements (P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu) in ready-to-eat “baby leaf” vegetables. The results obtained in our study for watercress microgreens are in good agreement with the values for “baby leaf” watercress reported by those authors. A recently published study by Pinto et al. (2015) compared the mineral element compositions of lettuce microgreens and mature lettuce grown under the same conditions and showed that lettuce microgreens possessed higher contents of most elements (Ca, Mg, Fe, Mn, Zn, Se and Mo) than the mature lettuces.

Previous studies (Khader and Rama, 2003; Santos et al., 2014) demonstrated that in addition to the intrinsic factors of microgreen species and varieties, the major sources of variability in nutrient composition and mineral content were extrinsic factors, such as soil composition, water availability, application of fertilizers, climatic conditions, seasonal variations, and maturity stages. In our study, plant maturity stage (i.e. growth period in days) would be the main extrinsic factor which affected the mineral content since all other extrinsic factors aforementioned were held constant by the grower.

Plant cells tend to accumulate K for essential functions such as cellular metabolism and stomatal opening and exclude Na, resulting in a high K/Na ratio in plant tissues (Flyman and Afolayan, 2008; Khader and Rama, 2003). Phosphorous and Zn assimilation are correlated with the initial stages of plant development (e.g., the synthesis of new protoplasm), whereas Ca and Fe are usually accumulated at more mature stages of growth or in more mature parts of plants. The results obtained from the 30 microgreen varieties tested in this study demonstrated that K is the principle mineral accumulated in all samples with a variable K/Na ratio ranging from 3.1 to 11.6. Phosphorous concentrations were higher than Ca concentrations in most of samples (66%) and Fe was always higher than Zn levels. These trends were also found in a previous study on baby greens (Santos et al., 2014).

3.2. Hierarchical cluster analysis in 10 genera

This study profiled mineral element components of microgreen samples in 30 varieties representing 10 species in 6 genera of the Brassicaceae family. Our data demonstrate that variation was present not only in overall mineral element contents among the 30 microgreen varieties, but also in the concentrations of the different mineral elements. We utilized two hierarchical cluster analysis methods (average-linkage distance between two clusters

and Ward's minimum variance in semi-partial R-square) to evaluate whether these trends were consistent across the 10 species examined (Fig. 1a and b, respectively). Both hierarchical cluster analyses provided the same prediction, showing that the 10 microgreen genera were classified into two groups. One group containing three species (*Raphanus sativus*, *Brassica oleracea*, and *Brassica napus*) was distinct from the other group represented by seven species (*Barbarea verna*, *Nastritum officinale*, *Brassica rapa*, *Lepidium bornariense*, *Brassica juncea*, *Eruca sativa*, and *Brassica narinosa*). The latter group was represented by one cluster with *Barbarea verna* and *Nastritum officinale* and a second cluster comprised of *Brassica rapa*, *Lepidium bornariense*, *Brassica juncea*, *Eruca sativa*, and *Brassica narinosa*. Cluster analysis groupings based on mineral element content were not an informative indicator of genus relatedness.

4. Conclusions

The determination of mineral elements by ICP OES provided a satisfactory quantification of Ca, Mg, P, K, Na, Fe, Zn, Mn, and Cu in 30 different varieties of microgreens in the Brassicaceae family. Samples were analyzed for the toxic heavy metals Cd and Pb, as well and neither of them was detected. The results of this study revealed that Brassicaceae microgreens are a good source of both macroelements (e.g., K and Ca) and microelements (e.g., Fe and Zn) in a balanced human diet and the consumption of microgreens could be a health-promoting strategy to meet the requirement of element dietary reference intakes, particularly for children.

Acknowledgements

The authors thank Fresh Origins for planting the microgreens samples for this study. Special thanks to Ms. Mebrat Gesese for her technical assistance throughout the work. Use of a company or product name by the U.S. Department of Agriculture does not imply approval or recommendation of the product to the exclusion of others that may also be suitable. This research was funded by USDA-ARS project no. 8042-43440-005D.

References

- Association of Official Analytical Chemists(AOAC), (1984). Official methods of analysis of the AOAC, Arlington, VA, 14th ed., Method 3.014(a), 38–40.
- Boeing, H., Bechthold, A., Bub, A., Ellinger, S., Haller, D., Kroke, A., Leschik-Bonnet, E., Müller, M.J., Oberritter, H., Schulze, M., Stehle, P., Watzl, B., 2012. Critical review: vegetables and fruit in the prevention of chronic diseases? *Eur. J. Nutr.* 51 (6), 637–663.
- Camaschella, C., 2015. Iron-deficiency anemia. *N. Engl. J. Med.* 372 (19), 1832–1843.
- dos Santos, I.F., dos Santos, A.M., Barbosa, U.A., Lima, J.S., dos Santos, D.C., Matos, G. D., 2013. Multivariate analysis of the mineral content of raw and cooked okra (*Abelmoschus esculentus* L.). *Microchem. J.* 110, 439–443.
- Flyman, M.V., Afolayan, A.J., 2008. Effect of plant maturity on the mineral content of the leaves of *Momordica balsamina* L. and *Vigna unguiculata* subsp. *Sesquipedalis* (L.) Verdc. *J. Food Qual.* 31 (5), 661–671.
- Hentze, M.W., Muckenthaler, M.U., Galy, B., Camaschella, C., 2010. Two to tango: regulation of mammalian iron metabolism. *Cell* 142 (1), 24–38.
- Hussain, J., Ali, L., Khan, A., Maboood, F., Gillani, S., Al-Harrasi, A., 2014. Nutritional assessment and mineral composition of some selected edible vegetables? *Eur. J. Med. Plants* 4 (4), 444–457.
- Kawashima, L.M., Valente Soares, L.M., 2003. Mineral profile of raw and cooked leafy vegetables consumed in Southern Brazil? *J. Food Compos. Anal.* 16 (5), 605–611.
- Khader, V., Rama, S., 2003. Effect of maturity on macromineral content of selected leafy vegetables. *Asia Pac. J. Clin. Nutr.* 12 (1), 45–49.
- Kopsell, D.A., Sams, C.E., 2013. Increases in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light emitting diodes. *J. Am. Soc. Hortic. Sci.* 138 (1), 31–37.
- Kou, L., Yang, T., Luo, Y., Liu, X., Huang, L., Codling, E., 2014. Pre-harvest calcium application increases biomass and delays senescence of broccoli microgreens. *Postharvest Biol. Technol.* 87, 70–78.
- Maathuis, F.J., 2009. Physiological functions of mineral macronutrients? *Curr. Opin. Plant Biol.* 12 (3), 250–258.
- Macdonald, H.M., New, S.A., Fraser, W.D., Campbell, M.K., Reid, D.M., 2005. Low dietary potassium intakes and high dietary estimates of net endogenous acid

- production are associated with low bone mineral density in premenopausal women and increased markers of bone resorption in postmenopausal women. *Am. J. Clin. Nutr.* 81 (4), 923–933.
- Martinez-Ballesta, M., Dominguez-Perles, R., Moreno, D., Muries, B., Alcaraz-López, C., Bastías, E., García-Viguera, C., Carvajal, M., 2010. Minerals in plant food: effect of agricultural practices and role in human health. A review. *Agron. Sustainable Dev.* 30 (2), 295–309.
- Nieves, J.W., 2005. Osteoporosis: the role of micronutrients. *Am. Soc. Clin. Nutr.* 81 (5), 1232S–1239S.
- Pinto, E., Almeida, A.A., Aguiar, A.A., Ferreira, I.M., 2015. Comparison between the mineral profile and nitrate content of microgreens and mature lettuces. *J. Food Compos. Anal.* 37, 38–43.
- Sanchez-Castillo, C.P., Dewey, P.J., Aguirre, A., Lara, J.J., Vaca, R., Leon de la Barra, P., Ortiz, M., Escamilla, I., James, W.P.T., 1998. The mineral content of Mexican fruits and vegetables. *J. Food Compos. Anal.* 11 (4), 340–356.
- Santos, J., Oliva-Teles, M., Delerue-Matos, C., Oliveira, M., 2014. Multi-elemental analysis of ready-to-eat baby leaf vegetables using microwave digestion and high-resolution continuum source atomic absorption spectrometry. *Food Chem.* 151, 311–316.
- Scherz, H., Kirchoff, E., 2006. Trace elements in foods: zinc contents of raw foods—a comparison of data originating from different geographical regions of the world. *J. Food Compos. Anal.* 19 (5), 420–433.
- Souzan, S.L., El-Aal, H.A., 2007. Minerals profile—shelf life extension and nutritive value of fresh green leafy vegetables consumed in Egypt. *Afr. Crop Sci. Conf. Proc.* 1817–1826.
- Sun, J., Xiao, Z., Lin, L.Z., Lester, G.E., Wang, Q., Harnly, J.M., Chen, P., 2013. Profiling polyphenols in five *Brassica* species microgreens by UHPLC-PDA-ESI/HRMSⁿ? *J. Agric. Food Chem.* 61 (46), 10960–10970.
- Xiao, Z., Lester, G.E., Luo, Y., Wang, Q., 2012. Assessment of vitamin and carotenoid concentrations of emerging food products: edible microgreens? *J. Agric. Food Chem.* 60 (31), 7644–7651.